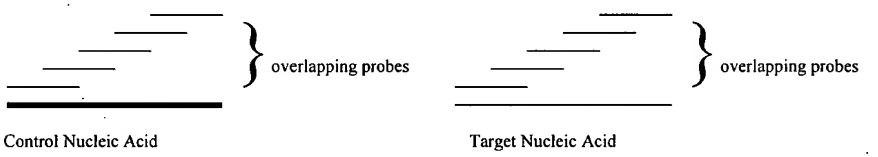
REMARKS

Claims 1-16 are pending in the instant application. Reconsideration of the claims in light of the amendments presented above and remarks that follow is respectfully requested.

The Invention

The instant invention is a method of determining the location and identity of differences in nucleotide sequence between a control nucleic acid and a target nucleic acid. The invention relies on differences in the melting temperatures of matched and unmatched nucleic acid hybrids to accomplish this determination. In one embodiment of the invention, the first step is to generate a set of overlapping probes that are complementary to a control nucleic acid. This step is outlined in (A) below. The melting temperatures for each of these probes and the control nucleic acid is then determined via methods well known in the art. The next step is to take this set of overlapping probes and determine each of their melting temperatures with a target nucleic acid in the same fashion. The ΔT_m can then be determined for each probe by calculating the difference, if any, between a probe's melting temperature with the control nucleic acid and that same probe's melting temperature with the target nucleic acid. Finally, the $\Delta\Delta T_m$ is determined by calculating the difference, if any, between the ΔT_m of one probe and the ΔT_m of an overlapping probe. If the $\Delta\Delta T_m$ is zero, there is no difference between the control nucleic acid and the target nucleic acid over the sequence encompassed by the overlapping probes. If the $\Delta\Delta T_m$ is non-zero, it is an indication of the location and identity of the nucleotide or nucleotides that are different between the control and target nucleic acids. Independent Claim 1 encompasses this aspect of the invention.

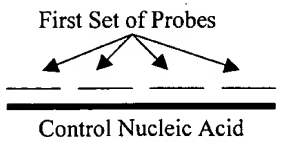
(A)

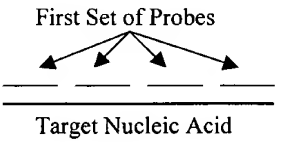


In a second embodiment of the invention, the oligonucleotides in a first set of probes do not overlap. Instead, these probes hybridize along the length of the control nucleic acid with at least one nucleotide intervening between the sites of hybridization, as shown in (B) below. The first step of this embodiment is to determine the melting temperature of these non-overlapping probes with the control nucleic acid. As above, the melting temperatures of these probes with the target nucleic acid are then also measured. The difference between these two T_m measurements is the ΔT_m for each of the probes in the first set. Unlike the above embodiment, determining the location and identity of nucleotide differences between the control and target nucleic acids in this second embodiment requires a second set of probes which overlap the sites of hybridization of the first set. This second set of probes is shown in (C) below. Identical melting temperature determinations are made for the second set of probes as were made for the first, i.e. between each of the second probes and the control and target nucleic acids, and again ΔT_m calculations can be made comparing an individual probe's T_m with the control and target nucleic acids.

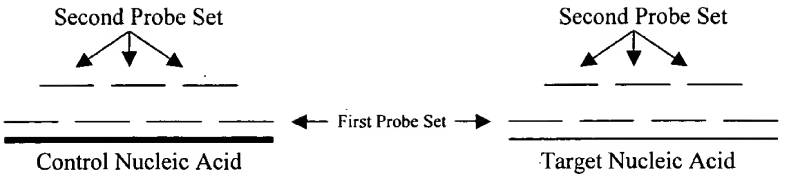
Nucleotide differences between the control and target sequences can be identified by calculating the $\Delta\Delta T_m$ of a probe from the first set and an overlapping probe from the second set. The $\Delta\Delta T_m$ determination is the same as is described above in connection with the first embodiment (these two probes would be identical to the overlapping probes in that embodiment). In addition, this second embodiment also easily allows one to determine if the site of the nucleotide difference is between two probes of the first set. For example, if the $\Delta\Delta T_m$ of two adjacent probes from the first set is zero then it can be concluded that there is no difference between the control and target nucleic acids over the regions those two probes hybridize. However, if a probe from the second set (that overlaps those initial probes) has a non-zero $\Delta\Delta T_m$ when compared to one or the other of the initial probes, then there must be a sequence alteration in the region covered by the three probes and thus this alteration must be between the two probes from the first set. Independent Claim 6 encompasses this aspect of the invention.

(B)





(C)



Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 1-16 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. In particular, the Examiner finds the recitation of "each of said at least two probes" in Claims 1-5 redundant. Applicant respectfully submits that Claims 1-5 do not include a recitation of "each of said at least two probes." Accordingly, withdrawal of this rejection is respectfully requested.

The Examiner also requests that Claims 1-16 clearly indicate that $\Delta\Delta T_{\underline{m}}$ is determined. Independent Claims 1 and 6 have been amended to clearly indicate that $\Delta\Delta T_m$ is determined. Accordingly, withdrawal of this rejection is respectfully requested.

The Examiner next requests clarification in Claims 6-16 regarding which set of probes hybridize to the target nucleic acid and which set hybridize to the control nucleic acid. As discussed in detail above, in order to calculate ΔT_m each probe of each set is hybridized to both the target nucleic acid and to the control nucleic acid. Accordingly, Applicants submit that Claims 6-16 are sufficiently definite, as currently amended, and withdrawal of this rejection is respectfully requested.

The Examiner also requests clarification in Claim 8 regarding whether the difference in ΔT_m between at least two overlapping probes indicates the location in the control nucleic acid of a nucleotide which is different from the target nucleic acid. As described in detail above,

determining the $\Delta\Delta T_m$, which is the difference in ΔT_m between at least two overlapping probes, indicates the location of a nucleotide difference between a control and a target nucleic acid. Accordingly, Applicants submit that Claim 8, as amended, is sufficiently definite and withdrawal of the rejection is respectfully requested.

Finally, the Examiner requests clarification in Claim 12 of how ΔT_m is determined based upon the two probes from said first set of probes and one probe of a second set of probes on the control nucleic acid. As described above, ΔT_m is determined for each individual probe and is the difference in melting temperature of a probe and the control and that probe and the target nucleic acid. Once ΔT_m is known for each of the 3 probes recited in Claim 12, $\Delta\Delta T_m$ may be determined (1) between the two probes of the first set; (2) between one probe of the first set and the probe of the second set; and (3) between the other probe of the first set and the probe of the second set. As described above, determining the $\Delta\Delta T_m$ of these combinations indicates the identity and location of a sequence alteration between the control and target nucleic acids.

Rejection under 35 U.S.C. § 103(a)

Claims 1-16 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Guo et al., Nature Biotechnology, Vol. 15, 331-335, 1997. In particular, the Examiner asserts that Guo et al. calculated $\Delta\Delta T_m$ and suggested that this could be used to identify a sequence alteration in a target nucleic acid. The Examiner bases this assertion on the determination by Guo et al. that the difference in melting temperature for 1 mismatch vs. 2 mismatches is larger than the difference in melting temperature for 0 mismatches vs. 1 mismatch. However, merely comparing the melting temperatures of two probes (or even three probes) is not a calculation of $\Delta\Delta T_m$, and would not allow one to determine the location or identity of a sequence alteration between a control and a target nucleic acid.

When rejecting a claim under 35 U.S.C. § 103, the Examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993). To establish a *prima facie* case the Examiner must show that the prior art reference, or references when combined, teach or suggest each and every limitation of the claimed invention. M.P.E.P. § 706.02(j). As discussed below, the Examiner has failed to establish that Guo *et al.* teach or

suggest each and every limitation of the claimed invention, and thus the Examiner has not established a *prima facie* case of obviousness in this case.

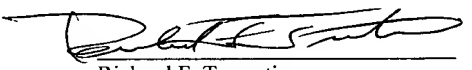
The two independent claims of the instant application each comprise four steps: (1) hybridization of probes to a target nucleic acid; (2) determining the melting temperature of those probes and the target nucleic acid; (3) determining the difference between the melting temperature of the probes and the target and the melting temperature of the probes and a control nucleic acid (the difference is termed "ΔT_m"; (4) determining the difference between the ΔT_m of two different probes (termed the "$\Delta\Delta T_m$"). As Guo *et al.* does not teach or suggest comparison of ΔT_ms for two probes, i.e. calculating the $\Delta\Delta T_m$, the Examiner has not carried her burden in establishing a *prima facie* case of obviousness and the rejection under 35 U.S.C. 103(a) should be withdrawn

CONCLUSION

On the basis of the remarks presented herein, Applicants believe that this application is now in condition for immediate allowance. Applicants respectfully request that the Examiner pass this application to issue, and early notice of such is requested. This paper is filed under 37 C.F.R. section 1.34(a).

Respectfully submitted,
DORSEY & WHITNEY LLP

Date: 10/15/03



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